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Interaction between temperature and photoperiod in regulation of flowering time in rice

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Photoperiod and temperature are two pivotal regulatory factors of plant flowering. The floral transition of plants depends on accurate measurement of changes in photoperiod and temperature. The flowering time of rice (*Oryza sativa*) as a facultative short-day (SD) plant is delayed under long-day (LD) and/or low temperature conditions. To elucidate the regulatory functions of photoperiod and temperature on flowering time in rice, we systematically analyzed the expression and regulation of several key genes (*Hd3a*, *RFT1*, *Ehd1*, *Ghd7*, *RID1*/*Ehd2*/*OsId1*, *Se5*) involved in the photoperiodic flowering regulatory pathway under different temperature and photoperiod treatments using a photoperiod-insensitive mutant and wild type plants. Our results indicate that the *Ehd1-Hd3a/RFT1* pathway is common to and conserved in both the photoperiodic and temperature flowering regulatory pathways. Expression of *Ehd1*, *Hd3a* and *RFT1* is dramatically reduced at low temperature (23°C), suggesting that suppression of *Ehd1*, *Hd3a* and *RFT1* transcription is an essential cause of delayed flowering under low temperature condition. Under LD condition, *Ghd7* mRNA levels are promoted at low temperature (23°C) compared with normal temperature condition (28°C), suggesting low temperature and LD treatment have a synergistic role in the expression of *Ghd7*. Therefore, upregulation of *Ghd7* might be a crucial cause of delayed flowering under low temperature condition. We also analyzed *Hd1* regulatory relationships in the photoperiodic flowering pathway, and found that *Hd1* can negatively regulate *Ehd1* transcription under LD condition. In addition, *Hd1* can also positively regulate *Ghd7* transcription under LD condition, suggesting that the heading-date of rice under LD condition is also regulated by the *Hd1-Ghd7-Ehd1-RFT1* pathway.

rice, photoperiod, temperature, flowering regulation, interaction

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Crop yields are strongly associated with flowering time. The floral transition of crops depends mainly on the accurate measurement of changes in day length (photoperiod) and temperature, which is regulated by both endogenous genes and environmental factors. Plants can perceive and respond to changes in photoperiod [1,2]. Recent molecular biological work reveals that the rice (*Oryza sativa*) genes *Heading date 3a* (*Hd3a*) and *Rice FT-like 1* (*RFT1*), orthologs of *Arabidopsis FLOWERING LOCUS T* (*FT*), encode florigens that can move from the leaf to the shoot apical meristem (SAM) and induce flowering in plants [3–5]. Under short-day (SD) conditions, expression of *Hd3a* promotes rice flowering by the *OsGI- Heading date 1*(*Hd1*)*-Hd3a* pathway which is conserved with the *GIGANTEA* (*GI*)*-CONSTANS* (*CO*)*-FT* pathway in *Arabidopsis* [3,6,7]. In this pathway, *Hd1* is an ortholog of *CO* in *Arabidopsis*, and encodes a transcription factor with a zinc finger domain, serving as a promoter of rice flowering under SD conditions and an inhibitor under long-day (LD)

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conditions [6,8,9]. The circadian clock-associated protein *OsGI* is an activator of *Hd1*, upregulating the expression of *Hd1* under SD conditions, and then upregulates the expression of *Hd3a* to finally promote flowering in rice [7,10]. *Early heading date 1* (*Ehd1*), which encodes a B-type response regulator, is an upstream gene of *Hd3a* and *RFT1* and upregulates the expression of these genes to promote flowering independently of *Hd1* under SD conditions [11]. *Rice Indeterminate1* (*RID1*)*/Ehd2/Oryza sativa Indeterminate1*(*OsId1*) is also a positive regulator of rice flowering, and can promote flowering by upregulating *Ehd1*, *Hd3a* and *RFT1* expression under both SD and LD conditions [12−14]. *OsMADS51* serves as another flowering promoter that can transmit a SD promotion signal from *OsGI* to *Ehd1, Hd3a* and *RFT1* [15].

Under LD conditions, rice flowering is delayed, and the *OsGI-Hd1-Hd3a* pathway is a suppression pathway. That is, *Hd1* inhibits *Hd3a* transcription to delay the heading-date of rice under LD conditions [16]. Another flowering repressor *Grain number, plant height, and heading date 7* (*Ghd7*), which encodes a transcription factor with a CCT motif, also acts to delay the flowering of rice under LD conditions by downregulating *Ehd1* mRNA levels [17]. This is the main suppression pathway under LD conditions. Also, *Se5*, encoding an important heme oxygenase enzyme involved in phytochrome chromophore biosynthesis, can inhibit the flowering time of rice under both SD and LD conditions [18,19]. Although rice flowering is delayed under LD conditions, it is still induced. It has been suggested that three transcription factors, *RID1/Ehd2/OsId1*, *OsMADS50* and *Ehd3*, are involved in rice floral induction under LD conditions. *RID1/Ehd2/OsId1* and *OsMADS50* can induce rice flowering by promoting *Ehd1* mRNA levels [12−14,20], while *Ehd3*, a protein with a PHD finger domain, can promote flowering under LD conditions by the downregulation of flowering repressor *Ghd7* mRNA levels [21]. In addition, recent studies have shown that *Hd3a* and *RFT1* serve different functions under different photoperiods [10,22]. *Hd3a* plays an important role in promoting flowering under SD conditions, while *RFT1* acts as a promoter under LD conditions [10,22].

Temperature also has an important effect on floral transition. Although it is well known that rice flowering is delayed under low temperature conditions, the molecular mechanisms involved in temperature response remain unknown. Our previous study investigated molecular regulation of rice flowering in different temperature conditions (27 and 23°C) and revealed that *Hd1* expression was not significantly changed. However, *Hd3a* mRNA levels were strongly suppressed by low temperature under both SD and LD conditions [23]. This result suggested that the temperature regulatory pathway for rice flowering might interact with the photoperiod regulatory pathway. In this study, to further reveal the molecular mechanisms of temperature response in rice flowering and analyze the interaction between the temperature and photoperiod pathways, we analyzed the expression patterns of several key genes involved in the photoperiodic flowering regulatory pathway under different temperature and photoperiod treatments using Real-time PCR. Our results showed that *Ehd1*, *Hd3a*, *RFT1*, *Ghd7* are shared and conserved in both the photoperiodic and temperature flowering regulatory pathways in rice. Several other genes, including *Hd1*, *Se5* and *RID1/Ehd2/OsId1*, have differentiated functions in the photoperiodic and temperature regulatory pathways.

1 Materials and methods

1.1 Plant materials

Two rice materials were used in this study, Zhonghua 11 (*Oryza sativa* L. ssp. *japonica*, wild type) and *lf1132*, a mutant derived from a tissue culture line of Zhonghua 11. The mutant has a loss of function allele for the *Hd1* gene, with two inserted fragments and several single-base substitutions at the *Hd1* locus [23]. Plants were grown in the experimental field of the Tianjin Agricultural Academy of Science (TAAS), Tianjin Province and at Sanya, Hainan Province.

1.2 Plant growth conditions in artificial climate cabinets

Zhonghua 11 and *lf1132* plants were sowed in natural fields in Tianjin, and strong seedlings were transferred at about 3 weeks old to artificial climate cabinets (MMM, Climacell, Germany). Four treatments mixing two photoperiods and two temperatures were used in the artificial climate cabinets: LD, 28°C; LD, 23°C; SD, 28°C; SD, 23°C. The photoperiod parameters were: LD, 14 h light and 10 h dark; SD, 10 h light and 14 h dark. The temperature parameters were: normal temperature, 28°C; and low temperature, 23°C.

1.3 Real-time PCR analysis

After 20 d in artificial climate cabinets, RNAs were isolated from leaves using Trizol solution (Invitrogen, USA) and treated with DNase I (NEB, USA). cDNAs were synthesized from 2 μg of total RNA using M-MLV reverse transcriptase (TaKaRa, Dalian, China). One microliter of cDNA was used for real-time PCR analysis using SYBR Green PCR master mix (Tiangen, Beijing, China) and performed in a My iQ™ 2 Two Color Real-Time PCR Detection System (Bio-Rad, USA). The gene-specific primers are shown in Table 1. Three replicates of each reaction were performed, and *OsActin* was used as an internal control for relative quantification of target gene expression. The amplification conditions were: 2 min at 95°C; then 40 cycles of 30 s at 95°C, 30 s at 59°C, and 30 s at 68°C.

Table 1 Primers used in this study

Primers	Sequences (5′–3′)
OsActin-F	GACTCTGGTGATGGTGTCAGC
OsActin-R	GGCTGGAAGAGGACCTCAGG
Hd3a-F	TTGGTAGGGTTGTGGGTGATGTGC
Hd3a-R	AGGTTAGGGTCACTTGGGCTTGGT
Ehd1-F	CGACAAAACACAAGACCACCCT
Ehd1-R	CCTGTTTGTCTGAATCCCATCG
RFT1-F	TCCGAGCCCAAGCAACCCTAAC
RFT1-R	AGTTCCTGGTGCTGAAGTTCTG
Ghd7-F	AGAGGAAGAAGAGGTGCTAC
Ghd7-R	GACATAGGTGGATGGCGGTG
Se5-F	GGAATACTGGGTTGGAGAGATC
Se5-R	CAGATTGCCCTCCCATTTGTAG
RID1/Ehd2/OsId1-F	AACAGCAGCAGCATCACTAC
RID1/Ehd2/OsId1-R	AGCAGGAGTGGTGGAGAATG

2 Results

2.1 The heading date of mutant and wild type in different regions

In our previous study, we analyzed the heading-date of mutant (*lf1132*) and wild type (Zhonghua 11) under different photoperiods (SD and LD conditions) in detail [23]. The heading-date of the mutant was almost identical under SD and LD conditions, while the heading-date of Zhonghua 11 was 21 d later under LD condition than SD condition [23]. This result suggested that the mutant was photoperiod-insensitive. Here, we further investigated the heading-date of mutant and wild type at different latitudes. We sowed wild type and mutant plants in Hainan (the lower latitude region, N 18°15′) on November 28, 2009 and in Tianjin (the higher latitude region, N 39°13′) on April 18, 2010. Days-to-heading of mutant and wild type were 61 and 70 d in Hainan, respectively, and 83 and 121 d in Tianjin (Figure 1A and B). The heading-date of the mutant was 9 d earlier than the wild type in Hainan Province. In contrast, the heading-date of the mutant was 38 d earlier in Tianjin (Figure 1A and B). To understand the difference in heading-date between these two areas, we further analyzed the changes in temperature and photoperiod during their growth stages in these two areas. According to the photoperiod curve, day-length changed from 10.5 h to 12.5 h during their development (from November to April) in Hainan, while day-length changed first from 12.5 h to 14.5 h, and then from 14.5 h to 10.5 h (from April to October) in Tianjin (Figure 1C). This means SD conditions were dominant in Hainan, while LD conditions were dominant in Tianjin. According to the temperature curve, the average temperature was higher from sowing to heading stage (from 20 to 28°C) in Hainan (Figure 1D), while the average temperature was below 22°C at the start of plant development (from April to the beginning of June) in Tianjin (Figure 1E). Photoperiod and temperature results together indicate that the delayed heading-date in Tianjin is mainly caused by LD and lower temperature conditions. The heading-date of the mutant was delayed 22 d in Tianjin compared with Hainan, while the wild type was delayed 51 d (Figure 1B). These results show that the higher latitude region (lower temperature and LD conditions) strongly delays the heading-date of the wild type. The difference between the two plants may be due to higher photoperiod sensitivity in the wild type compared with the mutant.

2.2 *Hd1* regulatory relationship analysis in the photoperiodic flowering pathway

The *lf1132* mutant has a loss of function allele of the *Hd1* gene with two inserted fragments and several single-base substitutions at the *Hd1* locus, and was identified and characterized in detail in our previous study [23]. Previous studies have demonstrated that *Hd1* acts as a core regulator of the photoperiodic flowering pathway, and can directly regulate *Hd3a* mRNA levels to promote rice flowering under SD conditions and delay rice flowering under LD conditions [3,16]. *Ehd1* can promote rice flowering independently of *Hd1* by inducing the expression of *FT*-like genes under SD conditions [11]. *RFT1* and *Hd3a* are functionally differentiated under SD and LD conditions [22]. However, there have been no direct results to reveal the regulatory relationships between *Hd1* and several key genes involved in the photoperiodic flowering pathway such as *Ehd1*, *RFT1*, *Ghd7*, *Se5* and *RID1/Ehd2/OsId1*. Here, we systemically analyzed the regulatory relationships between *Hd1* and these genes using an *Hd1*-deficient mutant. Under SD condition, the *Ehd1* transcript displayed the same expression patterns in the wild type and the mutant; a peak before the end of the dark phase and a trough before the end of the light phase (Figure 2A). The mRNA levels of *Ehd1* were slightly reduced in the mutant plants (Figure 2A). However, under LD condition, the *Ehd1* transcript was very low in the wild-type plants, but displayed distinct expression in the mutant plants (Figure 2B), suggesting that *Hd1* can negatively regulate *Ehd1* transcription. Previous studies have shown that *Ehd1* can promote rice flowering independently of *Hd1* deficiency under SD conditions [11], but it appears to be regulated by *Hd1* under LD conditions.

RFT1 is a homolog of the *Hd3a* gene, which has been studied extensively in previous reports [3,22]. Here, *RFT1* expression patterns were similar to *Hd3a* with a peak at the beginning of the light phase under SD condition, and its transcription level in the wild type was higher than in the mutant, indicating that *Hd1* can promote *RFT1* expression under SD condition (Figure 2C). Conversely, *RFT1* transcription in the mutant was higher than the wild type under LD condition, indicating that *Hd1* can inhibit *RFT1* tran-

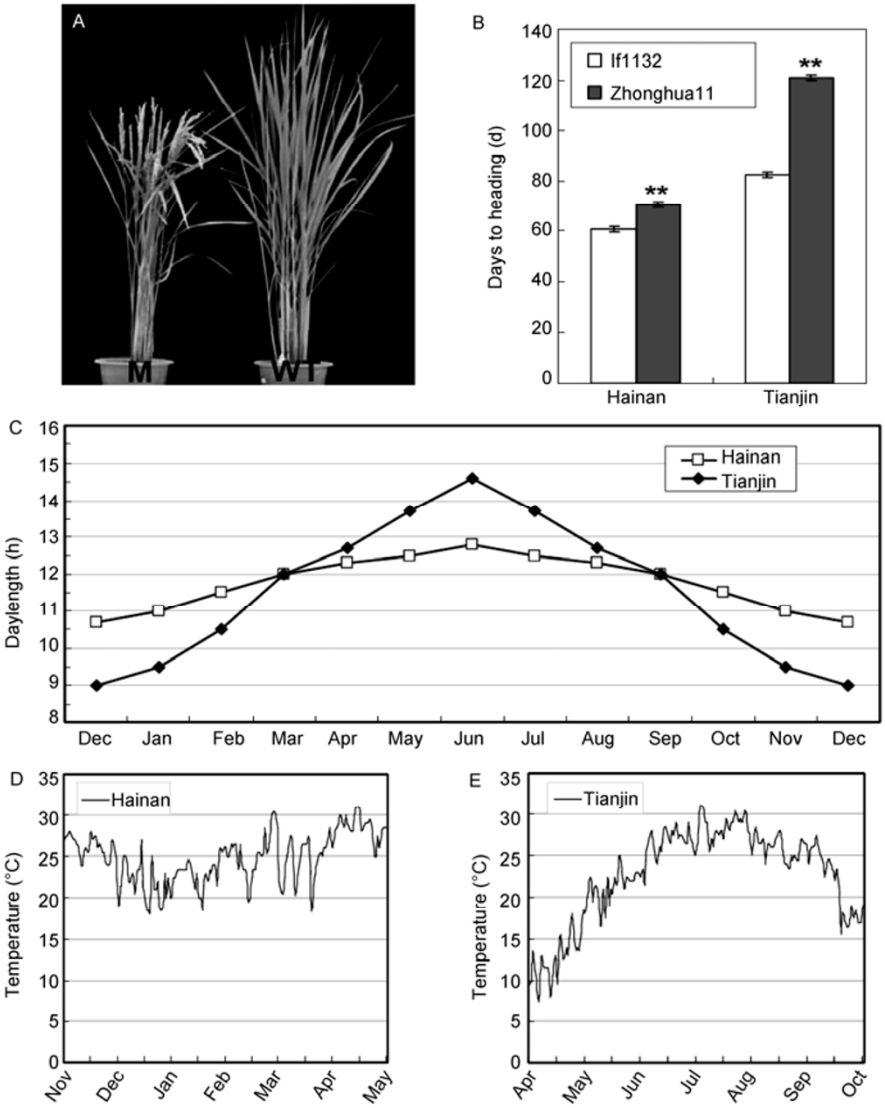

Figure 1 Analysis of heading-date in mutant and wild type plants in Hainan and Tianjin. A, The mutant displayed an early heading phenotype. M, mutant (*lf1132*); WT, wild type (Zhonghua11). B, The heading-date of the mutant and wild type in Hainan and Tianjin. The heading-date was recorded from 32 plants in both the mutant and wild type. C, Changes in photoperiod at Tianjin and Hainan. D and E, Changes in temperature (mean value of daily temperature) during the planting season at Hainan and Tianjin. A two-tailed Student's *t*-test was used to test the difference between two means: **, $P<0.01$.

scription under LD condition (Figure 2D).

We also analyzed the transcription levels of *Se5*, *RID1/Ehd2/OsId1* and *Ghd7*, which play important roles under LD conditions, in both wild type and mutant plants. The mRNA levels of *Se5* and *RID1/Ehd2/OsId1* were essentially unchanged in mutant compared with in wild type (Figure 2E and F). However, the mRNA levels of *Ghd7* in wild type plants were clearly higher than in the mutant, suggesting that *Hd1* can positively regulate *Ghd7* transcription under LD conditions (Figure 2G). According to this result and previous studies, the upregulation of *Ghd7* transcription by *Hd1* inhibits the transcription of *Ehd1* and *RFT1* to further delay rice heading-date under LD condition. Therefore, the heading-date of rice under LD condition is also regulated by the *Hd1-Ghd7-Ehd1-RFT1* pathway.

2.3 The *Ehd1-Hd3a/RFT1* pathway is common to and conserved in both the photoperiodic and temperature flowering regulatory pathways

To investigate the molecular mechanisms of the thermosensory pathway and the interaction between temperature and photoperiod in rice flowering, we measured the expression patterns of several genes involved in the photoperiodic flowering regulatory pathway in wild type and mutant plants under different temperature conditions. We first analyzed the expression of *Hd3a*. The mRNA levels of *Hd3a* were reduced in both wild type and mutant plants at the low temperature (23°C) compared with the normal temperature (28°C) (Figure 3A and B), which was consistent with our previous results [23]. We then analyzed the expression of *RFT1*, which is the closest homolog of *Hd3a*

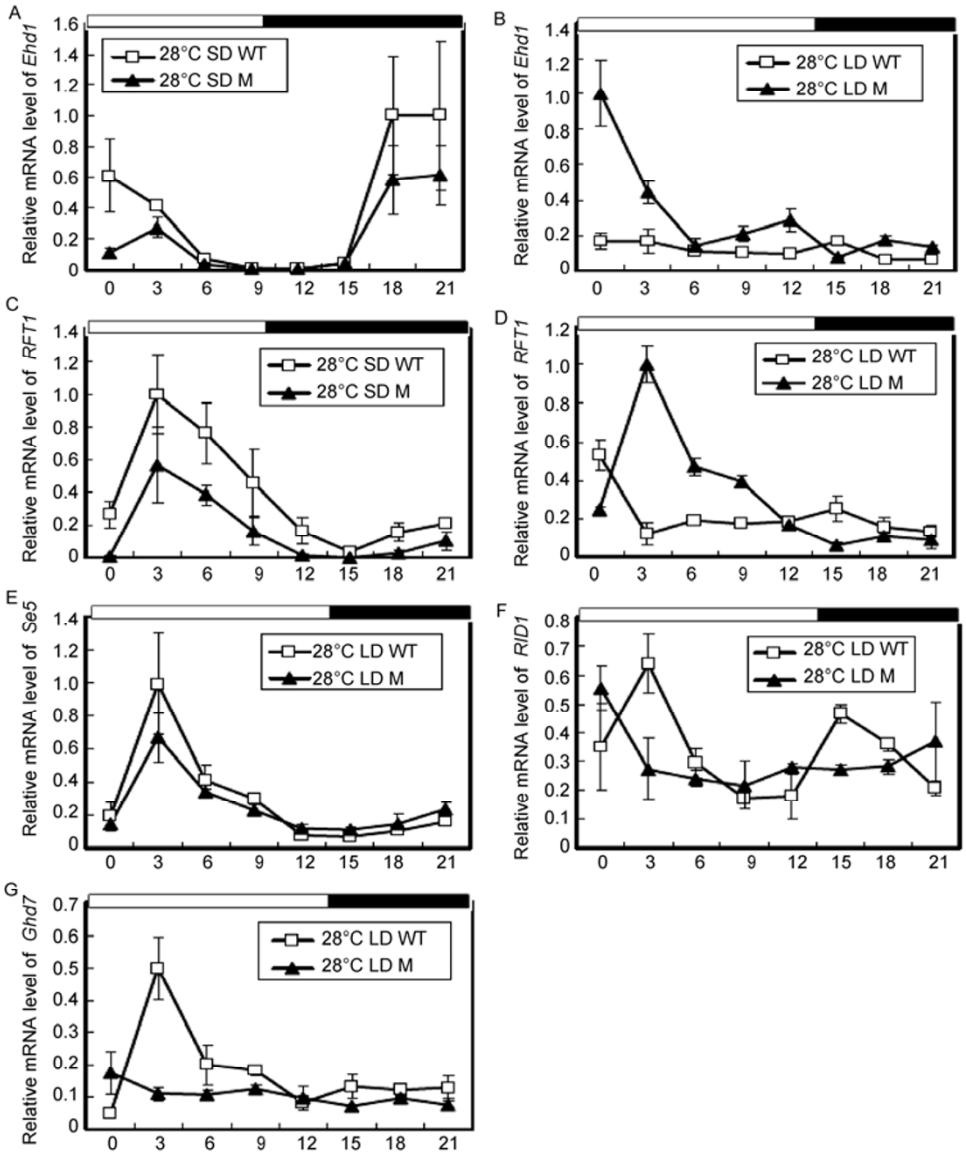

Figure 2 *Ehd1*, *RFT1*, *Se5*, *RID1/Ehd2/OsId1* and *Ghd7* expression in wild type and mutant plants under different photoperiods. Leaves were harvested from 40-day-old plants at the indicated times (once every 3 h for 24 h) grown in artificial climate cabinets, and Real-time PCR was carried out for analysis of *Ehd1*, *RFT1*, *Se5*, *RID1/Ehd2/OsId1* and *Ghd7* expression. M, mutant (*lf1132*); WT, wild type (Zhonghua 11). A and B, *Ehd1* expression profiles under SD and LD conditions; C and D, *RFT1* expression profiles under SD and LD conditions; E–G, *Se5*, *RID1/Ehd2/OsId1* and *Ghd7* expression profiles under LD conditions.

and regulated by *Hd1* and *Ehd1* [22]. Under SD condition, expression of *RFT1* in wild type and mutant plants was reduced at low temperature (23°C) compared with normal temperature condition (28°C) (Figure 3C and D), suggesting that low temperature has an important effect on *RFT1* transcription. Since *RFT1* is a major floral activator under LD conditions [22], we further analyzed the expression of *RFT1* under LD condition. Expression of *RFT1* under the low temperature (23°C) did not change significantly compared with the normal temperature (28°C) in either wild type or mutant plants (Figure 3E and F). This may be a result of interaction between LD conditions and low temperature. On the one hand, LD conditions and low temperature suppresses *RFT1* expression to delay rice flowering time; on the other hand, *RFT1* must maintain partial transcription to induce the floral transition under LD conditions. Therefore, LD conditions may have an "epistatic" effect on *RFT1* expression relative to the low temperature treatment.

Since *RFT1* and *Hd3a* are probably directly regulated by *Ehd1*, and *Ehd1* plays a main role under SD conditions (*Ehd1* transcription was very low under LD conditions) [11], we next analyzed the expression of *Ehd1* at different temperatures under SD condition. The mRNA levels of *Ehd1* in wild type and mutant plants were dramatically reduced under low temperature condition, to the point of being almost undetectable in wild type plants at low temperature (Figure

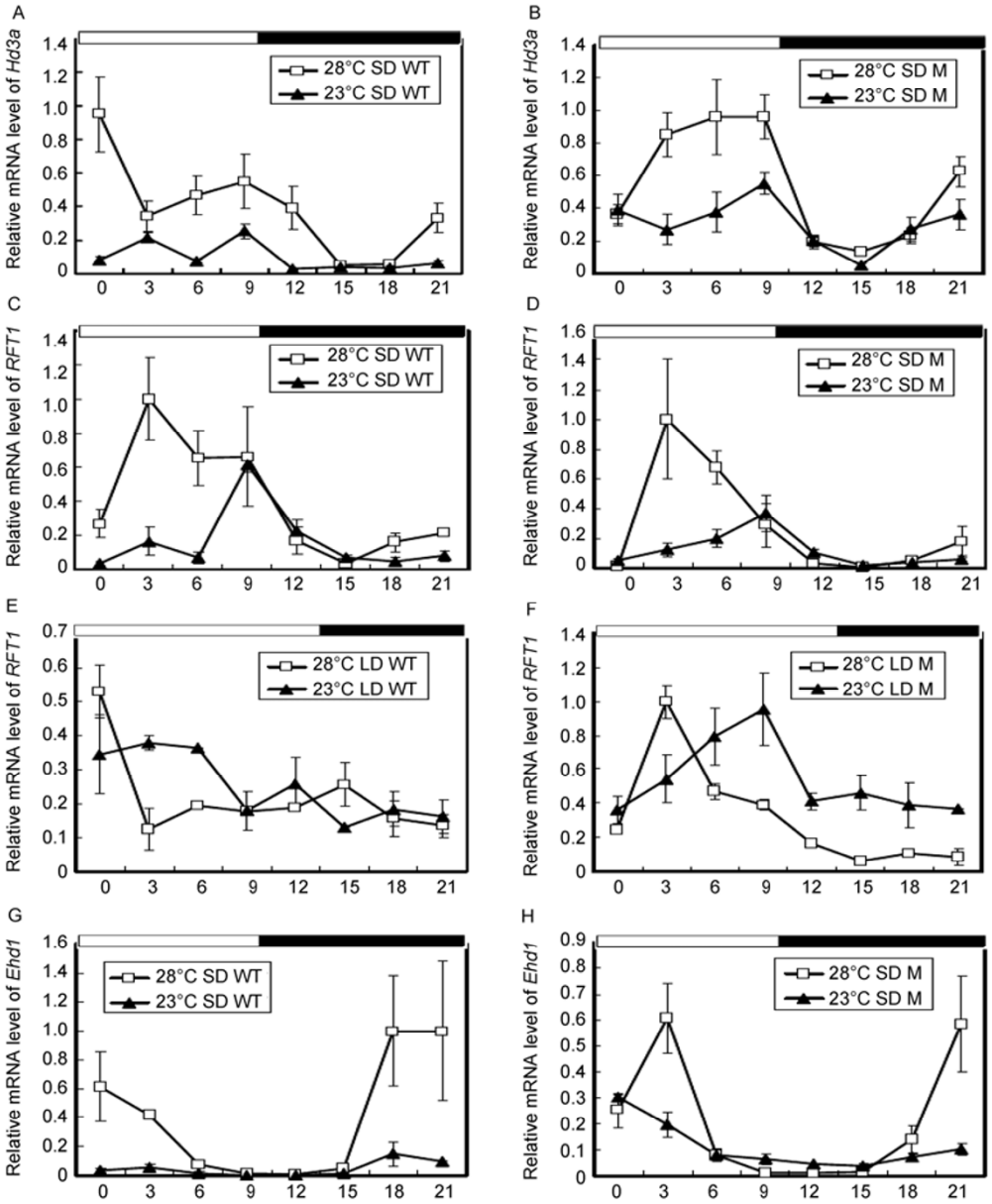

Figure 3 *Hd3a*, *RFT1* and *Ehd1* expression under different photoperiods and temperatures. Leaves were harvested from 40-day-old plants at the indicated times (once every 3 h for 24 h) grown in artificial climate cabinets, and Real-time PCR was carried out for analysis of *Hd3a*, *RFT1* and *Ehd1* expression. M, mutant (*lf1132*); WT, wild type (Zhonghua 11). A and B, *Hd3a* expression profiles at normal (28°C) and low temperature (23°C) under SD condition; A, wild type; B, mutant. C and D, *RFT1* expression profiles at normal (28°C) and low temperature (23°C) under SD condition; C, wild type; D, mutant. E and F, *RFT1* expression profiles at normal (28°C) and low temperature (23°C) under LD condition; E, wild type; F, mutant. G and H, *Ehd1* expression profiles at normal (28°C) and low temperature (23°C) under SD condition; G, wild type, H, mutant.

3G and H). Compared with *RFT1*, where partial expression was maintained, *Ehd1* and *Hd3a* transcription was very low, especially in wild type plants.

Taken together, *Ehd1*, *RFT1* and *Hd3a* expressions were all downregulated and suppressed under low temperature and LD conditions, and previous studies have shown that the *Ehd1-Hd3a/RFT1* pathway is essential in photoperiodic flowering regulation [2,3,5,10–12,22]. Therefore, we suggest that the *Ehd1-Hd3a/RFT1* pathway is common to and conserved in both the photoperiodic and temperature flowering regulatory pathways.

2.4 Low temperature has a significant effect on *Ghd7* transcription under LD condition

Ehd1, *RFT1* and *Hd3a* are located downstream of the photoperiodic flowering pathway, and based on the above results, are responsive to temperature change. In addition, our previous results showed that the mRNA level of *Hd1* upstream of the photoperiodic flowering pathway was not sig-

nificantly changed under low temperature condition [23]. Therefore, we analyzed the expression patterns of other genes upstream of the photoperiodic flowering under normal and low temperature conditions. We first investigated the mRNA levels of *Ghd7*, which plays an important role in the regulation of rice flowering time under LD conditions by downregulating *Ehd1* and *Hd3a* expression [17]. Under LD condition, *Ghd7* mRNA is much more abundant at low temperature than in normal temperature condition (Figure 4A), suggesting that low temperature has a significant effect on *Ghd7* transcription. This indicates that upregulation of *Ghd7* expression might be a crucial cause of delayed flowering in low temperature and LD conditions. We next analyzed the expression of *Ghd7* under SD condition. *Ghd7* transcription was essentially unchanged at low temperature (23°C) compared with the normal temperature (28°C) (Figure 4B).

We also examined the expression of two other upstream genes, *Se5* and *RID1/Ehd2/OsId1*. The expression of these genes did not significantly change in different temperature treatments under either SD or LD conditions (Figure 4C–F), suggesting that *Se5* and *RID1/Ehd2/OsId1* are not involved in the temperature flowering regulatory pathway. These results indicate that the *Ghd7-Ehd1-RFT1* pathway may be important in the thermosensory response controlling rice flowering.

3 Discussion

Photoperiod and temperature are two important environmental factors that regulate rice flowering. Several key genes involved in the photoperiodic flowering regulatory pathway have been identified and characterized in rice, including *Hd1*, *Hd3a*, *RFT1*, *Ehd1*, *Se5*, *RID1/Ehd2/OsId1*, *Ghd7*, *OsMADS50*, *DTH8/Ghd8*, and *Ehd3*. These genes play important roles in the SD promotion, LD suppression and LD induction flowering pathways [3,5,6,10–14,17,18, 20–22,24,25]. Genes involved in temperature regulation of flowering time have been identified in *Arabidopsis*, but

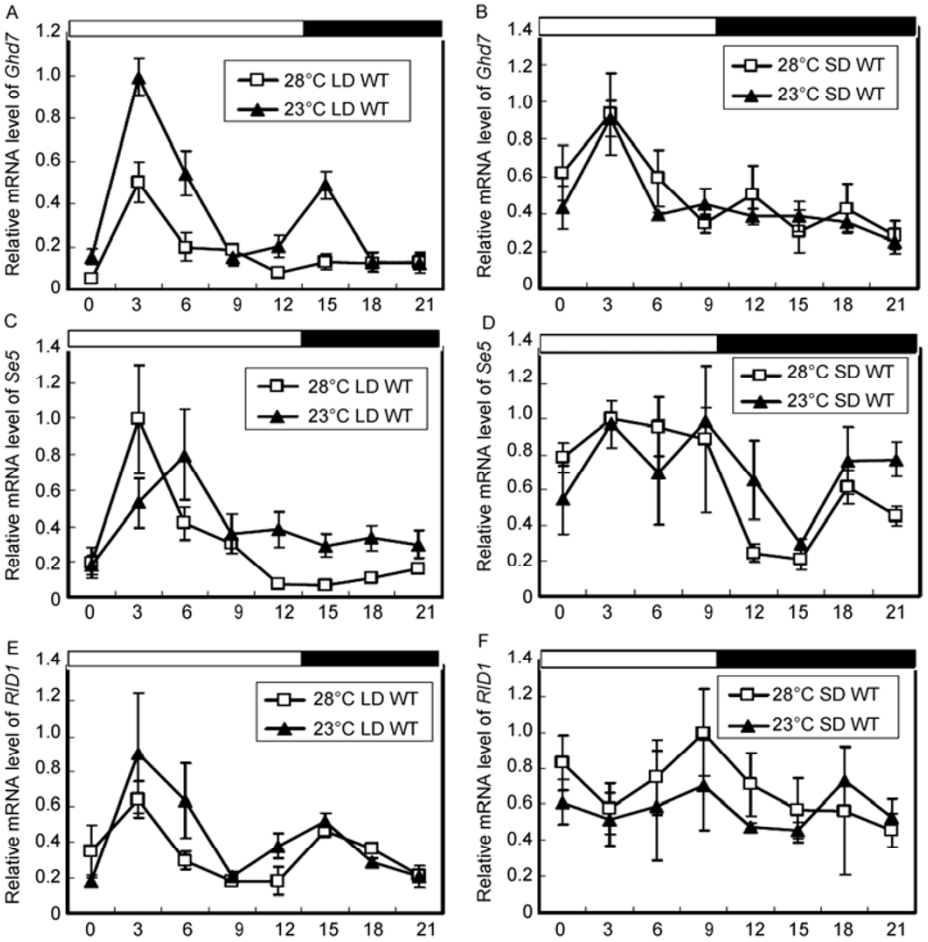

Figure 4 *Ghd7*, *Se5* and *RID1/Ehd2/OsId1* expression under different photoperiods and temperatures. Leaves were harvested at the indicated times (once every 3 h for 24 h) from 40-day-old plants grown in artificial climate cabinets, and Real-time PCR was carried out for analysis of *Ghd7*, *Se5* and *RID1/Ehd2/OsId1* expression. WT, wild type (Zhonghua 11). A and B, *Ghd7* expression profiles at normal and low temperature; A, under LD condition; B, under SD condition. C and D, *Se5* expression profiles at normal and low temperature; C, under LD condition; D, under SD condition. E and F, *RID1/Ehd2/OsId1* expression profiles at normal and low temperature; E, under LD condition; F, under SD condition.

little is yet known about this process in rice. In *Arabidopsis*, temperature can regulate flowering time by modulating the expression of the transcription factor *FLC* in the vernalization pathway [26–29]. The *Arabidopsis SHORT VEGETATIVE PHASE* (*SVP*) gene also plays an important role in temperature sensing by negatively regulating the expression of a floral integrator, *FT*, via direct binding to CArG motifs in the *FT* sequence [30], and loss of *SVP* function elicits insensitivity to ambient temperature changes. Recent results have also shown that nucleosomes containing the alternative histone H2A.Z are essential to perceiving ambient temperature in *Arabidopsis*. H2A.Z can confer distinct DNA unwrapping properties on nucleosomes to modulate the transcription of temperature-responsive regulators [31]. Unlike *Arabidopsis*, rice can flower without a vernalization treatment and rice genes homologous to *FLC* have not yet been found. *SVP*-group MADS-box proteins mainly work as negative regulators of brassinosteroid (BR) responses in rice and have no effect on flowering time [32]. Also, whether *Arabidopsis* H2A.Z is an evolutionarily conserved mechanism for temperature-sensing in rice is still unknown. Therefore, more work needs to be done to reveal the molecular mechanisms of temperature control of rice flowering.

In natural fields, rice cultivars have a wide distribution from Hainan in the south of China (~22°N) to Heilongjiang in the north (~45°N), and can flower and set seeds in these local regions. There are a few distributions even in high latitude cold regions such as Heihe in the northeast of China (~50°N) [33]. This wide distribution and adaptation of rice may be tightly associated with the conserved *Ehd1*-*Hd3a*/*RFT1* pathway. *RFT1* needs a balanced expression to induce to flowering in high latitude cold regions (LD and low temperature regions), that is, *RFT1* transcription is reduced to delay flowering time, but must be maintained at some level to induce the floral transition under these conditions. This balance may be regulated by upstream genes such as *Ghd7* and *Ehd1*.

Our previous results revealed that low temperature exerted a strong effect on the expression of *Hd3a*, which is downstream of the photoperiodic regulation pathway for flowering time in rice, indicating that temperature and photoperiod might interact or crosstalk in the gene regulatory network for rice flowering [23]. Here, we further investigated the expression of several important genes involved in the photoperiodic regulatory pathway under different temperature treatments. The results indicate that the expression of *Ehd1*, *RFT1* and *Ghd7* at low temperature is significantly different than under normal temperature conditions. *Ehd1* and *RFT1* transcription is downregulated and *Ghd7* transcription is upregulated under low temperature conditions, suggesting that *Ehd1*, *RFT1* and *Ghd7* are common to both the photoperiodic and temperature regulatory pathways. However, *Hd1* transcription is unchanged in different temperature treatments [23], suggesting that *Hd1* is not involved in the temperature flowering regulatory pathway. This result is similar to findings in *Arabidopsis*, where the thermosensory flowering pathway is mediated by *FT* (a homolog of *Hd3a* and *RFT1* in rice) expression levels, while *CO* (a homolog of *Hd1* in rice) is not essential for perceiving temperature [34]. *Se5* and *RID1*/*Ehd2*/*OsId1* transcriptions are also essentially unchanged in different temperature treatments. *Se5* encodes a key heme oxygenase enzyme involved in phytochrome chromophore biosynthesis [18], a component of photoperiod response. *RID1*/*Ehd2*/*OsId1* is exclusive to rice and does not have orthologous genes in *Arabidopsis* [12–14]. So far, no evidence exists that *Se5* and *RID1*/*Ehd2*/*OsId1* are involved in temperature response.

Based on our experimental results and previous studies, we would like to propose a primary model to explain the regulation of flowering time under different temperature and photoperiod conditions (Figure 5). Under SD conditions, 'X' proteins strongly suppress *Ehd1* and its downstream *Hd3a* and *RFT1* mRNA levels to delay rice flowering at low temperatures. So far, these upstream regulatory proteins are unknown. They may be specific to the temperature pathway. Under LD conditions, the upregulation of *Ghd7* expression at low temperatures suppresses the downstream *Ehd1* and *RFT1* mRNA levels to delay rice flowering, suggesting that the low temperature and LD treatments have a synergistic role in delaying rice flowering. It remains unclear whether unknown proteins (Ys) regulate *Ghd7* or *Ehd1* mRNA levels in low temperature conditions.

Hd1 and *Ehd1* are two crucial flowering regulators and integrators. *Hd1* has dual roles under SD and LD conditions and *Ehd1* plays a main role under SD conditions in the photoperiodic flowering regulatory pathway [6,11,16]. Our results demonstrate that *Hd1* has an important regulatory

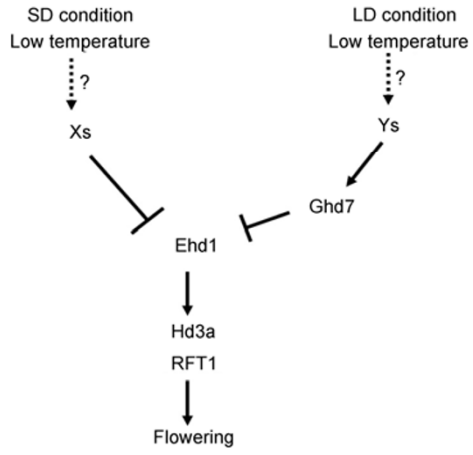

Figure 5 A model for the temperature control of flowering in rice under different photoperiod conditions. Under SD conditions, unknown proteins (Xs) may suppress *Ehd1* and its downstream *Hd3a* and *RFT1* mRNA levels to delay rice flowering at low temperatures. Under LD conditions, the upregulation of *Ghd7* expression by unknown proteins (Ys) suppresses the downstream *Ehd1* and *RFT1* mRNA levels to delay rice flowering.

function on *Ehd1* under LD conditions. *Ehd1* has an important effect on rice flowering in different temperatures, suggesting that *Ehd1* expression is thermosensitive and involved in the temperature flowering regulatory pathway. However, transcription of *Hd1* is essentially unaffected by different temperature treatments. These results suggest that temperature and photoperiod effects have differentiated pathways for *Hd1* gene regulation. So far, the upstream regulatory genes of *Ehd1* (except *Ghd7*) are still unknown in the temperature flowering regulatory pathway, and further study is required to improve our understanding of the gene network underlying temperature regulation of rice flowering.

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